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Repeated Testing of Rats Markedly Enhances the Duration of Effects Induced by Haloperidol on Treadmill Locomotion, Catalepsy, and a Conditioned Avoidance Response[1]

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HILLEGAART, V., S. AHLENIUS, O. MAGNUSSON AND C. J. FOWLER. *Repeated testing of rats markedly enhances the duration of effects induced by haloperidol on treadmill locomotion, catalepsy, and a conditioned avoidance response.* PHARMACOL BIOCHEM BEHAV 27(1) 159–164, 1987.—In the present experiments we have investigated the duration of haloperidol-induced effects in rats tested repeatedly, and in rats tested on a single occasion after drug administration. The behavioral test situations used include treadmill locomotion (speed 4 m/min), inclined grid catalepsy, two-way conditioned avoidance behavior and open field spontaneous locomotor activity. It was found that the duration of haloperidol-induced effects on treadmill locomotion, spontaneous locomotor activity, catalepsy and a conditioned avoidance response (doses: 0.32, 0.32, 1.25 and 0.2 mg/kg IP, respectively) was about 2 hr or less in animals observed once only ("independent procedure"). With the possible exception for locomotor activity, the duration of haloperidol-induced effects was considerably prolonged, up to 8 hr depending on the test situation, in animals observed repeatedly after the administration of haloperidol in the same doses ("dependent procedure"). The enhanced duration by the dependent procedure is probably not caused by handling stress since the enhanced duration of haloperidol-induced effects in some of the behavioral tests were only noted in animals tested repeatedly in one particular test, and not in animals given the repeated pretests in another situation. Furthermore, it was not possible to relate the enhanced duration of haloperidol-induced effects by repeated testing to changes in striatal DA metabolism as evaluated by measurements of DOPAC, HVA and DA. It was found that the treadmill test 2–4 hr after haloperidol (0.12–0.32 mg/kg IP), at certain doses and time intervals produced an increase in DA turnover [(DOPAC + HVA)/DA], but this increase was the same in both procedures (dependent and independent). Our findings that the time-course of action of haloperidol is dependent on the test procedures used may be of importance when interpreting interaction studies and the relationship between pharmacokinetics and behavioral effects of this, and possible other, psychotropic drugs.

Haloperidol Dopamine Test procedures Duration Rat

IN the establishment of the time-course of action of drugs, it is commonplace to make repeated observations of the same animals at several time points after drug administration, in order to record the first appearance of signs of drug effect, and thereafter to follow the animals until the effect has disappeared. In a recent experiment, examining interactions between some potential antipsychotic drugs, doses and time intervals were chosen according to a time-course established by repeated observations of the same animals. One of the reference compounds was haloperidol, where the repeated observations indicated a maximal effect on rat treadmill performance 2 hr after administration [3]. However, for animals observed at this time point only, there was no suppression of performance [9].

It has previously been reported that the duration of haloperidol-induced effects on catalepsy in rats is prolonged by repeated observations [20]. In continued studies on the effects of repeated testing on haloperidol-induced effects we thus included a test for catalepsy, as well as tests for treadmill performance, conditioned avoidance behavior and spontaneous locomotor activity in rats. Since haloperidol blocks central dopamine (DA) receptors [5], we also examined if the

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prolonged duration by repeated testing could be related to changes in brain DA turnover. Some animals were decapitated immediately after tests for treadmill performance, and the brain DA turnover was estimated by calculating the ratio between the DA metabolites dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and DA [(DOPAC + HVA)/DA].

METHOD

Animals

Adult male Sprague-Dawley rats (280–320 g) were used (ALAB Laboratorietjänst AB, Sollentuna, Sweden). The animals were kept under conditions of constant temperature (20–21°C), relative humidity (55–65%), and light-dark cycle (dark 6 a.m.–6 p.m.) Food (E3, Ewos, Södertälje, Sweden) and tap water was available ad lib. Experiments were performed between 9 a.m. and 6 p.m.

Drugs

Haloperidol (Janssen Leo Pharma AB, Helsingborg, Sweden) was dissolved in a drop of glacial acetic acid, and diluted to the appropriate volume with isotonic glucose (5.5%). The drug was injected IP in a volume of 2 ml/kg. Controls received the solvent vehicle alone.

Behavioral Measurements

Spontaneous locomotor activity and *rearing* were observed in a square open-field arena (680×680 mm), equipped with infra-red light sensitive photocells (for further details see [3]). *Treadmill* performance was observed on a rotating drum (ϕ=166 mm), at a speed of 8 rpm (4 m/min). The animals were trained to walk on the treadmill as previously described [3], and in the experiments the animals were observed for a maximal time of 2.5 min. *Catalepsy* was observed on an inclined grid (60°). Maximal observation time was 2.5 min. The scoring procedure, used in the evaluation of treadmill locomotion and catalepsy, assigns a score from 0–5 according to time (square transformation) as described in Ahlenius and Hillegaart [3]. It should be noted that control animals score 5 on the treadmill (i.e., they walk for the maximal time) and score 0 on catalepsy (i.e., display no immobility).

Conditioned avoidance behavior was observed in a conventional shuttle-box. The conditioned stimulus (CS) was provided by means of a white noise generator (Model 1501, Lafayette Instruments, Lafayette, IN). The unconditioned stimulus (UCS) was intermittent electric shocks, delivered via the grid floor of the shuttle-box. CS was presented for 10 sec followed by CS + UCS until the rat responded. The animals were trained to perform a conditioned avoidance response (CAR) in four daily sessions (20 trials/15 min). Experimental sessions consisted of 10 trials/7.5 min (for further details see [2]).

Test Procedures

The duration of the effects of haloperidol has been studied in the different test situations described above. Two different test procedures were used: (1) repeated observations of the same animals (dependent procedure), and (2) testing of naive animals at all time points (independent procedure). I.e., whereas in the dependent procedure one group of animals is injected with haloperidol and followed over time, in the latter procedure (independent) separate groups of animals were injected with haloperidol and observed after the various time intervals chosen.

Biochemical Measurements

Concentrations of DA, DOPAC and HVA were determined using high-performance liquid chromatography with electrochemical detection, as described previously [12]. Animals were killed by decapitation, and the brain was immediately removed. The neostriatum and the nucleus accumbens were dissected according to Ahlenius *et al.* [4].

Statistics

The appropriate parametric and non-parametric statistics [19,21] were used as indicated in the legends to figures and in the table.

RESULTS

Effects of Haloperidol on Animal Behavior: Effects of Test Procedure and Test Situation

Treadmill locomotion and catalepsy. There was a marked prolongation of the response to haloperidol in animals tested repeatedly for treadmill performance and catalepsy compared with animals tested once only (Fig. 1, middle and bottom). The duration of the effects of haloperidol (0.32 mg/kg) on treadmill performance was about 4 hr in animals tested repeatedly as compared with about 1 hr when independent measurements were made. The duration of the response to haloperidol (1.25 mg/kg) on catalepsy was 8 hr and 1 hr, respectively (for choice of doses see [3]). It should be noted that control animals tested repeatedly did not display any impairment in treadmill locomotion or any increase in catalepsy with time (see also [3]).

Locomotor activity. The duration of the effects of haloperidol (0.32 mg/kg) on locomotor activity appeared to be approximately the same (about 4 hr) regardless of the procedure followed (Fig. 1, top). Since repeated observations of rats in an open field results in habituation of the locomotor activity, the values obtained in animals tested repeatedly after haloperidol have been adjusted by means of a linear transformation, according to the decrease in activity displayed by controls observed repeatedly (data not shown) (see [8]).

Conditioned avoidance behavior. There was a marked statistically significant suppression of CAR performance 120 min after haloperidol administration (0.2 mg/kg) in animals previously tested at 30 and 60 min (dependent procedure) (Fig. 2). The rats tested 120 min after haloperidol only (independent procedure) also showed a slight but statistically significant reduction in CAR performance. However, there was a marked and statistically significant difference between the 2 hr performance in the two groups.

Effect of Repeated Treadmill Testing on the Duration of Effects by Haloperidol on Catalepsy and Vice Versa

In agreement with observations above, there was a marked prolongation of the effects of haloperidol on catalepsy or treadmill performance produced by repeated testing (Fig. 3). Thus, animals tested at 30 and 60 min were considerably more cataleptic and performed less well on the treadmill at 2 hr than animals tested at 2 hr only. However,

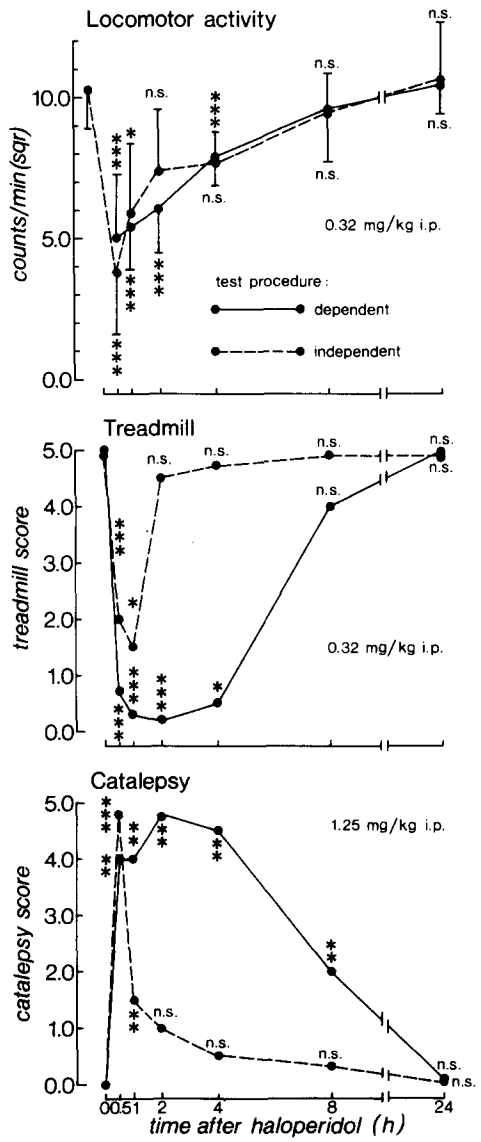

FIG. 1. Effects of haloperidol on locomotor activity, treadmill performance and catalepsy. Rats were observed either repeatedly at all time points shown in the figure (dependent procedure), or once only at a single time point (independent procedure). The data are presented as means±S.D. (locomotor activity) or medians (treadmill, catalepsy) (n=10 in all groups). Statistical analysis was performed by means of the Kruskal-Wallis one-way ANOVA followed by the Mann-Whitney U-test or the Friedman two-way ANOVA followed by the Wilcoxon matched-pairs signed-ranks test for comparisons with glucose-treated controls (treadmill and catalepsy) (n=10 in all groups). Locomotor activity data were analysed by means of a one-way ANOVA followed by Dunnett's *t*-test for comparisons with glucose-treated controls (independent procedure) or by means of Student's *t*-test for comparisons with controls at corresponding time intervals (dependent procedure). The values in the dependent procedure were adjusted for the habituation of locomotor activity that occurred in glucose-treated controls, by means of a linear transformation [8]. *Locomotor activity* (dependent): F(1,18) (treatment)=0.05, n.s.; F(5,90) (time)=39.12, $p<0.001$; F(5,90) (treatment × time)=1.13, n.s.; (independent): F(5,54)=6.16, $p<0.001$. *Treadmill* (dependent): $\chi^2(6)=32.91$, $p<0.001$; (independent): H(6)=24.64, $p<0.001$. *Catalepsy* (dependent): $\chi^2(6)=31.96$, $p<0.001$; (independent): H(6)=31.70, $p<0.001$. $^{n.s.}p>0.05$, $^{*}p<0.05$, $^{**}p<0.025$, $^{***}p<0.01$.

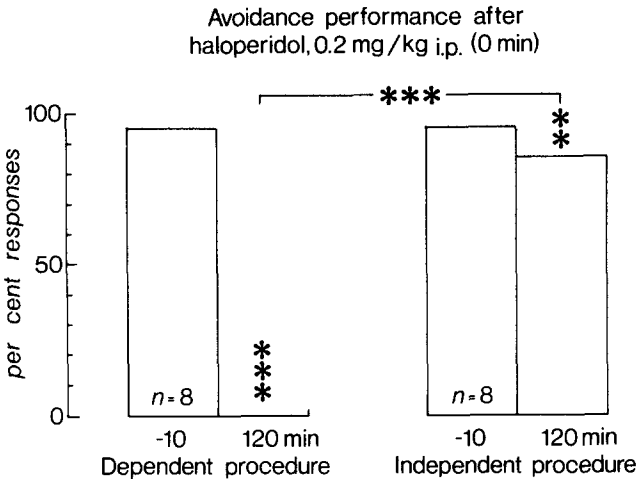

FIG. 2. Effects of haloperidol on a conditioned avoidance response. The rats were tested 30, 60 and 120 min after haloperidol (0.2 mg/kg IP) (dependent procedure) or only once at 120 min (independent procedure). The figure shows the median performance in a pre-test, and 120 min after the injection. Statistical analyses were undertaken using the Wilcoxon matched-pairs signed-ranks test for comparisons with pre-test performance and the Mann-Whitney U-test for comparisons between the two groups at 120 min. $^{**}p<0.025$, $^{***}p<0.01$.

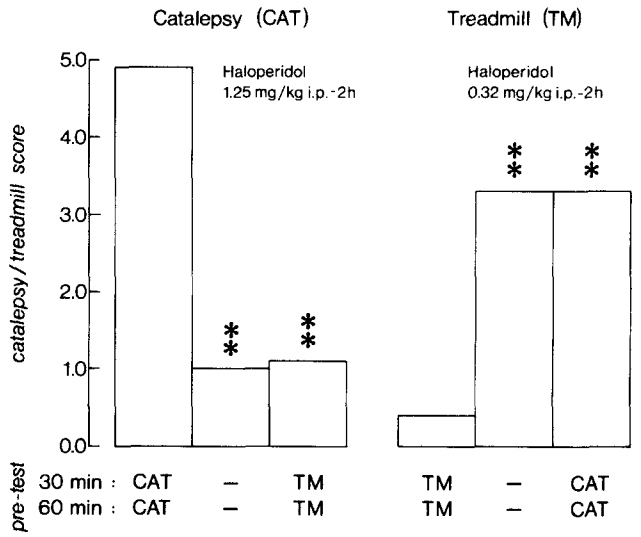

FIG. 3. Effects of treadmill pre-tests on final catalepsy performance and vice versa, after treatment with haloperidol. The animals were observed in two pre-tests as indicated at the bottom of the figure, and shown are the medians based on the performance of 8–9 animals per group for catalepsy or treadmill tests at 2 hr. Statistical comparisons were undertaken using the Kruskal-Wallis one-way ANOVA, followed by the Mann-Whitney U-test. *Catalepsy:* H(2)=14.82, $p<0.001$; *Treadmill*: H(2)=15.70, $p<0.001$. $^{**}p<0.025$.

the duration of haloperidol-induced effects on catalepsy or treadmill performance was not enhanced by previous tests in the other modality (Fig. 3). As shown in the figure, treadmill testing at 30 and 60 min did not affect the degree of catalepsy at 2 hr and vice versa.

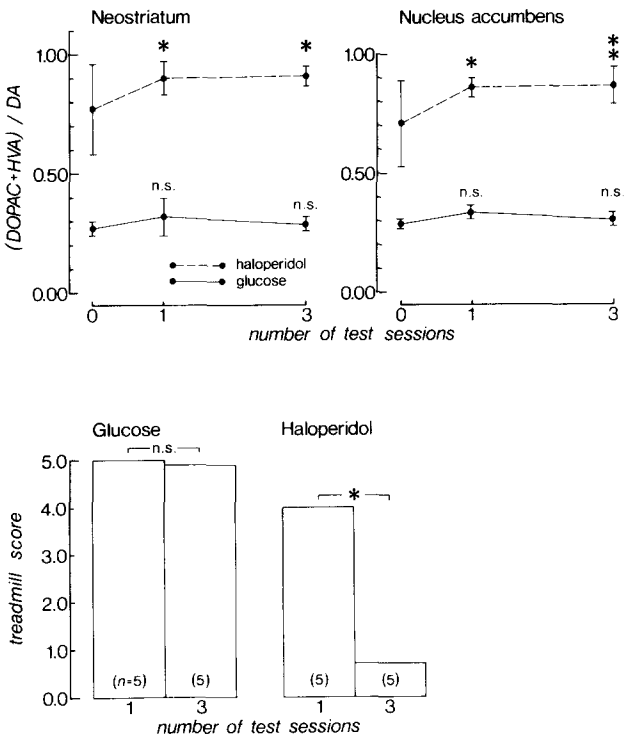

FIG. 4. Effects of number of pre-tests on final treadmill performance and on dopamine turnover after treatment with haloperidol. All animals were trained to walk on the treadmill according to the procedure described in the Method section before being given glucose or haloperidol (0.32 mg/kg IP). Controls, not tested on treadmill ("0 test sessions"), were decapitated at 2 hr for biochemical measurements. The other groups were decapitated at 2 hr after treadmill observations as indicated in the bottom part of the figure, i.e., the animals were observed either at 30, 60 and 120 min ("3 test sessions") or at 120 min only ("1 test session"). Statistical analysis of biochemical measurements by means of a two-way ANOVA, followed by *t*-tests for comparisons with controls (0 test sessions). *Neostriatum*: $F(1,24)$ (treatment)$=288.94$, $p<0.001$; $F(2,24)$ (test sessions)$=2.58$, n.s.; $F(2,24)$ (treatment × test sessions)$=1.28$, n.s. *Nucleus accumbens:* $F(1,24)=294.74$, $p<0.001$; $F(2,24)=4.01$, $p<0.05$; $F(2,24)=1.82$, n.s. Statistical analysis of treadmill performance was undertaken using the Mann-Whitney U-test. $*p<0.05$, $**p<0.01$.

Effects of Repeated Treadmill Testing on Striatal Dopamine Turnover

There was a marked increase in DA turnover in the neostriatum as a result of haloperidol treatment, as well as in the nucleus accumbens, in agreement with the literature (e.g., [14]). Furthermore, there was an increase in DA turnover as a result of treadmill testing (see Fig. 4). However, this effect was only observed in animals treated with haloperidol, where the increase in DA turnover was the same in animals tested once only as in animals tested repeatedly. Corresponding changes were obtained in the nucleus accumbens. Thus, no support for a difference in striatal DA turnover as an explanation for the differences in treadmill performance between animals tested once only and those tested repeatedly was obtained (Fig. 4, bottom).

As described above, the duration of haloperidol-induced effects on treadmill performance after repeated testing appears to be about 4 hr as compared to 1 hr in animals tested once only (see Fig. 1). In a series of experiments we have further evaluated time and dose-dependency of this phenomenon as well as effects of haloperidol on striatal DA turnover.

As shown in Table 1 (upper part), a dose of 0.32 mg/kg was needed to produce an enhanced duration of haloperidol-induced effects by repeated testing (2 hr). In this series of experiments, maximal effects produced by repeated testing were obtained 3 hr after haloperidol administration. Biochemical measurements of neostriatal DA turnover, however, could not be related to the behavioral effects obtained by haloperidol (Table 1, bottom part). In fact, the increase in neostriatal DA turnover, as a result of treadmill testing, appeared to be more pronounced at the lowest dose (0.12 mg/kg) and at the longest time interval after haloperidol 0.32 mg/kg (4 hr). No statistically significant enhancement of DA turnover was obtained at the dose and time-interval where maximal behavioral effects by haloperidol were found (3 hr). At this dose and time-interval after haloperidol, the highest increase in DA turnover was obtained, as compared with glucose-treated controls (data not shown), indicating the possibility that a ceiling effect was reached.

DISCUSSION

In agreement with a previous report by Stanley and Glick [20], it was found in the present study that the duration of the effects induced by haloperidol on catalepsy in rats were markedly enhanced by repeated testing. Although Stanley and Glick found differences in the degree of catalepsy shown 2 hr after haloperidol depending on the number of tests (up to 12), it is clear from the present experiments that as few as two tests (at 30 and 60 min) clearly affected the catalspsy score at 2 hr. In addition, a similar influence by repeated testing was also found on treadmill performance, and on a conditioned avoidance response (see Fig. 2). In fact, at the doses of haloperidol chosen (0.32 and 1.25 mg/kg for treadmill performance and catalepsy, respectively), a maximal effect on the behavior at 2 hr by repeated testing was found, whereas animals receiving haloperidol, but tested at 2 hr only, did not differ significantly from saline treated controls. Essentially the same results were obtained on a conditioned avoidance response test, although in this case there was a small albeit statistically significant effect of haloperidol in the animals tested at 2 hr only.

Costall *et al.* [6], comparing effects of repeated and single catalepsy tests after haloperidol administration, found no difference in the intensity of catalepsy between the two test procedures. This finding is not necessarily in conflict with the findings in the present study. However, in contrast to our results, they found no difference in the duration of haloperidol-induced effects depending on the test procedure used. One possible explanation for the divergent results is that Costall *et al.* [6] apparently lost sensitivity due to the use of a submaximal dose of haloperidol.

The experiments on locomotor activity did not show any significant prolongation of the duration of haloperidol-induced effects after repeated testing of the animals. However, in contrast to the tests for treadmill performance, catalepsy and conditioned avoidance behavior, there was an habituation by the animals to the test situation. Thus, in the presentation of the results we have made a correction for the habituation by means of a linear transformation (see e.g, [8]). It should be noted however, that there was a slight albeit not statistically significant effect at 2 hr in the direction predicted

TABLE 1

TREADMILL PERFORMANCE AND CONCOMITANT MEASUREMENTS OF NEOSTRIATAL DOPAMINE TURNOVER IN ANIMALS TESTED ONCE ONLY OR REPEATEDLY: TIME- AND DOSE-DEPENDENT EFFECTS OF HALOPERIDOL

	Time					
	2 hr		3 hr		4 hr	
	Number of Tests					
Haloperidol (mg/kg)	1×	3×	1×	6×	1×	8×
	Treadmill Performance (percent of controls)					
0.12	100	100	—	—	—	—
0.20	100	100	—	—	—	—
0.32	98	18*	96	4*	100	100
	(DOPAC + HVA)/DA (percent of controls)					
0.12	138*	127*	—	—	—	—
0.20†	112	120*	—	—	—	—
0.32	117*	118*	95	98	125*	123*

The treadmill performance scores have been expressed as percent of the pre-test control values (5.0 in all cases). Statistical comparisons were undertaken using the Wilcoxon matched-pairs signed-ranks test. (DOPAC + HVA)/DA measurements have been expressed as percent of haloperidol treated controls (not tested on the treadmill). Statistical evaluation was undertaken using a one-way ANOVA, followed by t-tests for individual comparisons.

*$p<0.05$ in comparison with glucose-treated controls (all other comparisons n.s.).

†Nucleus accumbens.

TABLE 2

EFFECTS OF HALOPERIDOL ON TREADMILL PERFORMANCE: POSSIBLE STATE-DEPENDENT EFFECTS

	Day 1				Day 3	
	Time (min)				Time (min)	
	−10	30	60	120	−10	120
Dependent	5.0	4.0	2.3	1.8 *	4.9	3.7 n.s.
Independent	5.0	—	—	4.6 *	4.8	3.5 n.s.

Haloperidol, 0.32 mg/kg IP was administered at time "0 min," and the animals were tested on the respective day as indicated in the table. Shown are the medians based on the performance of 10 animals tested repeatedly (dependent test procedure), and 12 animals tested only in a pretest, and at 2 hr, on the respective day (independent test procedure). Statistical comparisons between the performance of the two groups at the 2 hr time points were performed by means of the Mann-Whitney U-test.

n.s. $p>0.05$, * $p<0.05$.

from the results obtained in the other test situations. Similar results were also obtained when the values in dependent groups were adjusted with respect to the expected percent decrease, as evidenced by the habituation in controls tested repeatedly at corresponding time intervals (data not shown). Needless to say, the locomotor activity measurements allow far more variability of the animals than the other test situations, and therefore we can not exclude that further experiments will show effects by repeated testing also in this situation.

It is known that handling is stressful to rats as evidenced by elevated plasma levels of corticosterone (see e.g., [1,7]). It has further been speculated that changes in plasma steroid levels may change receptor sensitivity (e.g., [13,17]), a possibility considered in the experiments shown in Fig. 3. In these experiments animals were tested twice on treadmill and thereafter the degree of catalepsy was observed, or vice versa. These experiments indicated that the prolonged duration of the effects of haloperidol found after repeated testing are limited to the particular test situation, and not due to general effects of the experimental procedures like handling. These results also exclude the possibility that such general effects would produce changes in plasma levels of haloperidol resulting in different drug effects depending on the test procedure employed.

In a separate experiment we examined the possibility that state dependent effects (see e.g., [16]) might explain the findings with haloperidol in the present study. The findings presented in Figs. 1 and 3 could be interpreted in support of this explanation. As shown by the preliminary results presented in Table 2, however, we obtained no evidence for state-dependent effects.

The biochemical experiments showed the expected increase in striatal DA turnover by haloperidol treatment, and there was a further significant increase in the turnover by treadmill testing, but this increase was the same in animals tested repeatedly as in animals tested only once at 2 hr. Thus, we obtained no evidence for differences in striatal DA turnover as an explanation for differences in the behavioral effects of haloperidol in animals tested repeatedly as compared with animals given a single test. Furthermore, there is no simple correlation between changes in brain DA turnover as estimated by DOPAC, HVA and DA measurements, and treadmill performance. Thus, a maximal increase in striatal DA turnover was obtained at the lowest dose of haloperidol used (0.12 mg/kg), whereas no behavioral effects were noted by repeated testing after haloperidol with this dose. Similarly at 4 hr, when the behavioral effects obtained by repeated testing after haloperidol (0.32 mg/kg) had disappeared, there was still an increase in striatal DA turnover. This lack of correlation between biochemistry and behavior is in stark contrast to the very tight correlation between the effects of DA antagonists on apomorphine-induced stereotypies and their effects on DA turnover [11,18]. The concentrations of haloperidol used to produce the behavioral effects investigated in the present study are, however, higher than needed to antagonize apomorphine-induced stereotypies, and it is possible that a "ceiling" effect on DA turnover precludes any correlations. It should be noted that in the present experiments all animals were tested prior to decapitation. There is a possibility that the test as such produces an increase in DA turnover that overshadows any differences due to different pre-test schedules. In fact, we have some preliminary measurements on animals tested 30 and 60 min after haloperidol (0.32 mg/kg), decapitated, but not tested, at 2 hr. These animals appear to have a higher DA turnover than animals not tested at all, and decapitated at 2 hr. Experiments are in progress further to examine this possibility.

The present results not only have significance for the screening models used in analysis of the duration of potential antipsychotic compounds, but are also of relevance in other respects. For example, it is generally considered that there is a

poor correlation between the plasma levels of antipsychotic drugs and their antipsychotic activity (see [15]), and it has been suggested that in experimental animals, brain concentrations provide a better indicator of antipsychotic activity than plasma levels [10,15]. However, it is clear from the present experiments that, in addition to possible differences between plasma and brain levels of haloperidol, drug-behavioral interactions provide an important source of variability in a pharmacokinetic analysis of haloperidol-induced effects. In fact, independent, rather than dependent, observations appear to provide a much better means for obtaining correlations between tissue levels of haloperidol, and suppression of a conditioned avoidance response, when comparing the present data with those of Öhman *et al.* [15].

In conclusion, the present experiments demonstrate that repeated testing of animals may prolong haloperidol-induced effects in a number of test situations. It is obvious that such effects would influence a pharmacokinetic analysis, and it is possible that these findings, to some extent, may explain reported difficulties in making correlations between plasma levels of antipsychotic drugs and clinical efficacy. In the present experiments, it was not possible to relate the prolonged duration of haloperidol-induced behavioral effects to changes in striatal DA turnover as reflected by DOPAC,HVAand DA measurements.

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